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# Complement activity of polysaccharides from three different plant parts of *Terminalia macroptera* extracted as healers do

Yuan-Feng Zou [a,*], Bing-Zhao Zhang [a,b], Kari Tvete Inngjerdingen [a], Hilde Barsett [a], Drissa Diallo [c], Terje Einar Michaelsen [a], Berit Smestad Paulsen [a]

[a] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, P. O. Box 1068 Blindern, 0316 Oslo, Norway
[b] GIAT-HKU joint Center for Synthetic Biology Engineering Research (CSynBER), Guangzhou Institute of Advanced Technology, Chinese Academy of Sciences, Nansha, Guangzhou 511458, PR China
[c] Department of Traditional Medicine, BP 1746, Bamako, Mali

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ABSTRACT

*Ethnopharmacological relevance:* Water decoctions of the root bark, stem bark and leaves of *Terminalia macroptera* are used by traditional healers in Mali to cure a wide range of illnesses, such as wounds, hepatitis, malaria, fever, cough and diarrhea as well as tuberculosis. Plant polysaccharides isolated from crude water extracts have previously shown effects related to the immune system. The aims of this study are comparing the properties of the polysaccharides among different plant parts, as well as relationship between chemical characteristics and complement fixation activities when the plant material has been extracted as the traditional healers do, with boiling water directly.

*Materials and methods:* Root bark, stem bark and leaves of *Terminalia macroptera* were extracted by boiling water, and five purified polysaccharide fractions were obtained by anion exchange chromatography and gel filtration. Chemical compositions were determined by GC of the TMS derivatives of the methyl-glycosides and the linkage determined after permethylation and GC–MS of the derived partly methylated alditol acetates. The bioactivity was determined by the complement fixation assay of the crude extracts and purified fractions.

*Results:* The acidic fraction TRBD-I-I isolated from the root bark was the most active of the fractions isolated. Structural studies showed that all purified fractions are of pectic nature, containing rhamnogalacturonan type I backbone. Arabinogalactan type II side chains were present in all fractions except TRBD-I-II. The observed differences in complement fixation activities among the five purified polysaccharide fractions are probably due to differences in monosaccharide compositions, linkage types and molecular sizes.

*Conclusion:* The crude extracts from root bark and stem bark have similar total activities, both higher than those from leaves. The root bark, leaves and stem bark are all good sources for fractions containing bioactive polysaccharides. But due to sustainability, it is prefer to use leaves rather than the other two plant parts, and then the dosage by weight must be higher when using leaves.



## 1. Introduction

*Terminalia macroptera* Guill. & Perr. (Combretaceae) is a tree which occurs widely in West Africa. In Mali *Terminalia macroptera* is used against a variety of ailments, about 31 different indications have been mentioned by the traditional healers in ethnopharmacological studies. The stem bark and leaves are most commonly used against sores and wounds, pain, cough, tuberculosis and hepatitis (Pham et al., 2011a). The roots are used against hepatitis, gonorrhea and various infectious diseases, including *Helicobacter pylori*-associated diseases (Pham et al., 2011a; Silva et al., 1996, 1997, 2000, 2012). Flavonoids (Nongonierma et al., 1987, 1988, 1990), triterpenoids (Conrad et al., 1998, 2001a), ellagitannins (Pham et al., 2011b) and related phenolics (Conrad et al., 2001a, 2001b; Silva et al., 2000), have been identified from different parts of *T. macroptera*.

Water decoctions of *Terminalia macroptera*, administered orally, are the most common preparations used by the traditional healers

*Abbreviations:* AG-I, arabinogalactan type I; AG-II, arabinogalactan type II; Ara, arabinose; ASE, accelerated solvent extraction; BWE, boiling water extraction; Gal, galactose; GalA, galacturonic acid; Glc, glucose; GlcA, glucuronic acid; Man, mannose; RG-I, rhamnogalacturonan type I; Rha, rhamnose; Xyl, xylose

* Corresponding author. Tel.: +47 22856549; fax: +47 22854402.
*E-mail address:* yuanfeng.zou@farmasi.uio.no (Y.-F. Zou).

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in Mali (Pham et al., 2011a). Thus the boiling water extracts (BWE) should contain bioactive compounds present in the plant material. Plant polysaccharides isolated from crude water extracts have shown effects related to the immune system by different *in vitro* and *in vivo* test systems (Paulsen and Barsett, 2005). The chemical characteristics and biological activities of polysaccharides, especially those from plants used in the treatment of wounds, ulcer and cancer have been reported (Austarheim et al., 2012a; Lin et al., 2013; Samuelsen et al., 1996; Yamada and Kiyohara, 1999; Zong et al., 2012).

The root bark, stem bark and leaves of the tree are used frequently in traditional African folk medicine. If the root bark from a tree is collected this can lead to serious damages to the tree being greater than if the stem bark or leaves are collected. Therefore, in this study, BWE was employed to extract polysaccharides from root bark, stem bark and leaves from *Terminalia macroptera* for testing if all plant material contains bioactive polysaccharides of more or less equal bioactivity. Generally, studies of plant polysaccharides take place after the plant material has been extracted with organic solvent in order to remove lipids and low molecular weight compounds. The aims of this study are comparison of the polysaccharides properties among different plant parts, as well as relationship between chemical characteristics and complement fixation activities when the plant material has been extracted as the traditional healers do, with boiling water directly. Crude polysaccharide extracts were obtained and further purified, the chemical characteristics and complement fixation activities of polysaccharide fractions were evaluated, and the results from the three different plant parts were compared.

## 2. Materials and methods

### 2.1. Plant material

The root bark, stem bark and leaves of *Terminalia macroptera* were collected in Mali, and identified by the Department of Traditional Medicine (DMT), Mali. A voucher specimen is deposited at the herbarium of DMT (Voucher no. 2468/DMT). The plant material was washed, cut into small pieces, dried and pulverized to a fine powder by a mechanical grinder.

### 2.2. Extraction of polysaccharides

BWE was carried out in the way traditional healers in Mali make water decoctions. 200 g of powdered root bark, stem bark and leaves were weighed and placed in a pot, and extracted twice with boiling distilled water (2 L followed by 1 L) for 30 min each time. The extracts were centrifuged and filtered through Whatman no. 1 filter paper. The filtrates were combined and subjected to ultrafiltration with cut off 5000 Da, and the high molecular weight (HMW) fraction was subjected to dialysis in a dialysis tube with cut off 3500 Da, lyophilized and kept for further studies. The crude, dialyzed, water extracts were denominated TRBD for root bark extracts, TSBD for stem bark extracts and TLD for leaves extracts (Fig. 1). These fractions were subjected to monosaccharide determination, evaluation of presence of starch and the complement fixation assay; methods see below.

### 2.3. Fractionation and characterization of polysaccharides

The crude extracts showing high activity in the complement assay were further fractionated by anion exchange and gel filtration. All purified fractions were subjected to determination of their chemical and biological characteristics.

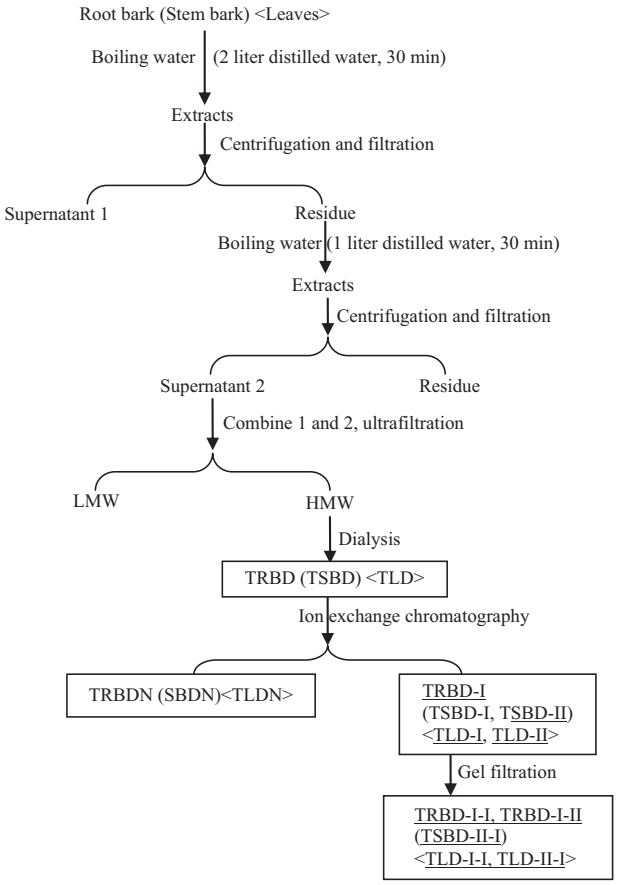


**Fig. 1.** Extraction and fractionation scheme of polysaccharides extracted with boiling water (BWE) from root bark, stem bark or leaves of *Terminalia macroptera* (underlined acidic fractions showed high complement fixation activity and were fractionated for further studies).

#### 2.3.1. Ion exchange chromatography and gel filtration

The crude extracts from BWE were filtered through 0.45 μm filters and applied to an anion exchange column packed with ANX Sepharose™ 4 Fast Flow (high sub) (GE Healthcare). The neutral fractions were eluted with distilled water at (2 mL/min), while the acidic fractions were eluted with a linear NaCl gradient in water (0–1.5 M) at 2 mL/min. The carbohydrate elution profiles were monitored using the phenol-sulfuric acid method (Dubois et al., 1956). The related fractions were pooled, dialyzed at cut-off 3500 Da against distilled water for removal of NaCl, and lyophilized.

The acidic fractions marked in Fig. 1 were dissolved in elution buffer (10 mM NaCl), filtered through a Millipore filter (0.45 μm), and subjected to gel filtration after application on a Hiload™ 26/60 Superdex™ 200 prep grade column (GE Healthcare) combined with the Äkta system (FPLC, Pharmacia Äkta, Amersham Pharmacia Biotech). Fractions were pooled based on their elution profiles, as determined by the phenol-sulfuric acid method, dialyzed and lyophilized.

#### 2.3.2. Determination of monosaccharide composition

The monosaccharide compositions of the crude extracts and purified fractions were determined by gas chromatography of the trimethylsilylated (TMS) derivatives of the methyl-glycosides obtained after methanolysis with 3 M hydrochloric acid in anhydrous methanol for 24 h at 80 °C (Austarheim et al., 2012b; Barsett et al., 1992; Chambers and Clamp, 1971). Mannitol was used as an internal standard. The TMS derivatives were analyzed by

capillary gas chromatography on a Focus GC (Thermo Scientific, Milan, Italy).

### 2.3.3. Test for the presence of starch

The presence of starch in the fractions was tested by adding two drops of an aqueous iodine-potassium-iodide solution to the samples (Hunter et al., 1970). A positive reaction gives a dark bluish color. Starch was used as a positive control.

### 2.3.4. Molecular weight determination

The homogeneity and molecular weight of the purified polysaccharide fractions were determined by size exclusion chromatography on a Hiload™ 16/60 Superdex™ 200 prep grade column (GE Healthcare) combined with the Äkta system (FPLC, Pharmacia Äkta, Amersham Pharmacia Biotech). Dextran polymers (Pharmacia) B512 (5.6 kDa), T8360 (19 kDa), T250 (233 kDa) and T500 (475 kDa) were used as calibration standards. Approximately 5 mg of the samples were dissolved in 2 mL of 10 mM NaCl buffer and filtered through a Millipore filter (0.45 μm) and applied to the column. The samples were eluted with 10 mM NaCl at 0.5 mL/min, collecting 2 mL fractions. The eluent was detected with a Shimadzu RI detector. The retention volume was converted to molecular weight by using the standards.

### 2.3.5. Precipitation with the Yariv β-glucosyl reagent

Precipitation with the Yariv β-glucosyl reagent was performed on the samples as described by van Holst and Clarke (1985). The Yariv β-glucosyl reagent forms a colored precipitate with compounds containing AG-II structures. A solution of Arabic gum in water (1 mg/mL) was used as a positive control.

### 2.3.6. Determination of phenolic content

The total amount of phenolic compounds in the purified polysaccharide fractions were quantitatively determined using the Folin–Ciocalteu assay (Singleton and Rossi, 1965). 200 microliter sample (1 mg/mL) dissolved in water (three replicates) was added the same amount of Folin–Ciocalteu's phenol reagent (1:1 in water, Merck/Kebo), mixed and left for 3 min at room temperature. 200 μL of 1 M $Na_2CO_3$ was added; the tubes were mixed and allowed to stand for 1 h. The absorbance was measured at 750 nm. A standard curve was plotted using ferulic acid. The total phenolic content was determined as ferulic acid equivalents (FA/sample) *x* 100%.

### 2.3.7. Determination of protein content

The protein content of the polysaccharide fractions was determined by the Bio-Rad protein assay, based on the method of Bradford (Bio-Rad, CA, USA; Bradford, 1976). The standard procedure for microtiter plates was used with bovine serum albumin (BSA) as a protein standard in a concentration range of 15–500 μg/mL. The Bio-Rad protein assay is a dye-binding assay in which a differential color change of a dye occurs in response to various concentrations of protein. The absorbance maximum for an acidic solution of Coomassie® Brilliant Blue G-250 dye shifts from 465 nm to 595 nm when binding to protein occurs.

### 2.3.8. Determination of glycosidic linkages

Glycosidic linkage elucidation was performed by methylation studies. Prior to methylation, the free uronic acids were reduced with $NaBD_4$ to their corresponding neutral sugars. After reduction of the polymers, methylation, hydrolysis, reduction and acetylation (Kim and Carpita, 1992) were carried out. The derivatives were analyzed by GC–MS using a GCMS-QP2010 (Shimadzu) attached to a Restek Rxi-5 MS (30 m; 0.25 mm i.d.; 0.25 μm film) column. The injector temperature was 280 °C, the ion source temperature 200 °C and the interface temperature 300 °C. The column temperature was 80 °C when injected, then increased with 10 °C/min to 140 °C, followed by 4 °C/min to 210 °C and then 20 °C/min to 300 °C. Helium was the carrier gas (pressure control: 80 kPa.) The compound at each peak was characterized by an interpretation of the retention times and the characteristic mass spectra. The estimation of the relative amounts of each linkage type was related to the total amount of each monosaccharide type as determined by methanolysis. Effective carbon-response factors were applied for quantification (Sweet et al., 1975).

### 2.3.9. Complement fixation assay

The complement fixation test is based on inhibition of hemolysis of antibody sensitized sheep red blood cells (SRBC) by human sera as described by Michaelsen et al. (2000) (Method A). Austarheim et al. (2012a) showed that endotoxin has no influence on this complement fixation assay. BPII, a highly active pectic polysaccharide from the aerial parts of *Biophytum petersianum* Klotzsch (Grønhaug et al., 2011), was used as a positive control. Inhibition of lysis induced by the test samples was calculated by the formula $[(A_{control} - A_{test})/A_{control}] \times 100\%$. From these data, a dose–response curve was created to calculate the concentration of test sample giving 50% inhibition of lysis ($ICH_{50}$). A low $ICH_{50}$ value means a high complement fixation activity. The activity of all the polysaccharide fractions are given as the $ICH_{50}$ value of the positive control BP-II divided on the $ICH_{50}$ value of the sample.

## 3. Results

### 3.1. Crude extracts

The crude extracts were obtained by boiling water extractions as described in Fig. 1. The yield of the crude extracts TRBD was highest, followed by TSBD and TLD, as showed in Table 1. All of the yields given in Table 1 are related to the dried, powdered plant materials.

After methanolysis and TMS-derivatisation of the obtained methylglycosides, the monosaccharide compositions of the crude extracts were determined by GC analysis. As can be seen from Table 1 the monosaccharide compositions of these extracts show the presence of the same monosaccharides, but the quantitative composition varies between the samples. All of the crude extracts contain the neutral monosaccharides arabinose (Ara), rhamnose (Rha), galactose (Gal) and glucose (Glc). In addition to these neutral monosaccharides, the crude extracts TRBD, TSBD and TLD also contain galacturonic acid (GalA). The content of Glc most

**Table 1**
Monosaccharide compositions of crude extracts from root bark, stem bark and leaves of *Terminalia macroptera*.

| | TRBD | TSBD | TLD |
|---|---|---|---|
| Ara[a] | 4.8 | 21.9 | 31.7 |
| Rha | 28.8 | 11.4 | 5.2 |
| Xyl | 2.6 | 2.6 | Trace |
| Man | n.d. | 0.6 | 1.2 |
| Gal | 7.2 | 18.6 | 12.6 |
| Glc | 53.8 | 22.2 | 20.5 |
| GlcA | Trace | 2.8 | 1.3 |
| GalA | 2.8 | 19.8 | 27.7 |
| Yield (% w/w)[b] | 8.4 | 5.5 | 4.3 |
| Presence of starch | + | + | + |
| Total activity[c] | 0.58 | 0.26 | 0.12 |

[a] mol% related to total content of the monosaccharides Ara, Rha, Xyl, Man, Gal, Glc, GlcA and GalA.
[b] Yield related to the dried, powdered materials.
[c] Total activity were calculated as yield × ($1/ICH_{50}$ value-sample).

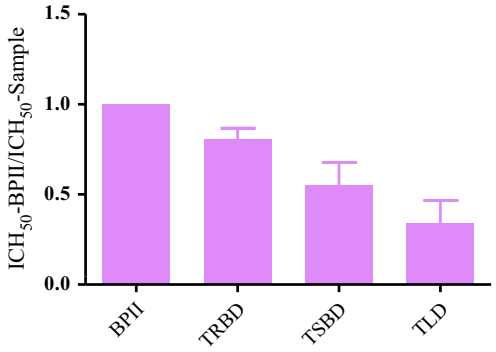


**Fig. 2.** Complement fixation activities of the crude extracts obtained by BWE from root bark, stem bark and leaves of *Terminalia macroptera* related to positive control (BPII from *Biophytum petersianum*). $ICH_{50}$-BPII/$ICH_{50}$-Sample shows how active each individual test sample is compared to the positive control.

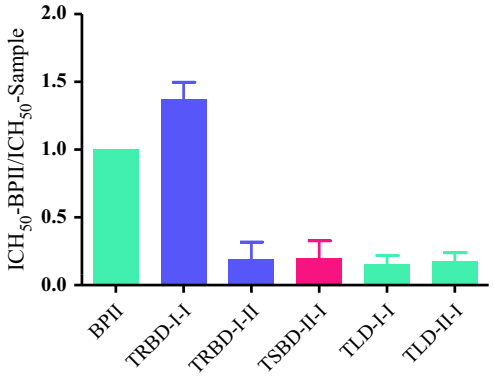


**Fig. 3.** Complement fixation activities of purified polysaccharide fractions obtained from root bark, stem bark and leaves of *Terminalia macroptera* related to positive control BPII. $ICH_{50}$-BPII/ $ICH_{50}$-Sample shows how active each individual test sample is compared to the positive control BPII.

**Table 2**
Characterizations of polysaccharide fractions isolated from root bark, stem bark and leaves of *Terminalia macroptera* after ion exchange chromatography and gel filtration.

| | TRBD-I-I | TRBD-I-II | TSBD-II-I | TLD-I-I | TLD-II-I |
|---|---|---|---|---|---|
| Ara[a] | 14.6 | 11.5 | 12.3 | 35.5 | 13.0 |
| Rha[a] | 17.6 | 10.4 | 13.4 | 5.4 | 10.6 |
| Xyl[a] | 5.9 | 6.2 | 3.8 | n.d. | 0.4 |
| Man[a] | 2.7 | 2.7 | 1.7 | n.d. | 0.4 |
| Gal[a] | 20.6 | 18.5 | 14.2 | 31.4 | 8.9 |
| Glc[a] | 2.4 | 27.1 | 4.1 | 1.3 | 1.6 |
| GlcA[a] | 3.9 | 3.0 | 4.2 | n.d. | 5.4 |
| GalA[a] | 32.4 | 20.7 | 46.2 | 26.3 | 59.6 |
| Yield (% w/w)[b] | 0.01 | 0.01 | 0.041 | 0.001 | 0.01 |
| Mw (kDa) | 71.5 | 5.5 | 23.3 | 115.9 | 23.3 |
| The Yariv test[c] | ++ | − | + | + | ++ |
| Presence of starch | − | + | − | − | − |
| Protein (% w/w) | 0.4 | 0.6 | 0.5 | 0.1 | n.d. |
| Phenols (% w/w)[d] | n.d. | 30.8 | 3.1 | 0.9 | 0.4 |

n.d. not detected.

[a] mol% related to total content of the monosaccharides Ara, Rha, Xyl, Man, Gal, Glc, GlcA and GalA.

[b] Yield related to the dried, powdered material.

[c] The presence of arabinogalactans type II (AG-II) was identified by precipitation with the β-glycosyl Yariv reagent.

[d] The total phenolic content is expressed as ferulic acid equivalents.

probably comes from starch as the iodine test gave a strong positive reaction (Table 1). The TRBD extract differ from the two other extracts by the lack of mannose (Man) and GalA, and in the TL extract no xylose (Xyl) was detected.

The complement system is an important part of the immune defense, such as the primary defense against bacterial invasions and viral infections. Complement fixating activity of polysaccharides from plants has previously been shown as an indicator for effects on the immune system (Inngjerdingen et al., 2013; Michaelsen et al., 2000).

As can be seen from Fig. 2, the crude extracts from BWE showed potent human complement fixation activities *in vitro*. The activity of the crude extract TRBD was higher than that from TSBD and TLD, all having slightly lower activities than the positive control BPII. As shown in Table 1, the total activity of the crude extract TRBD from root bark was higher than those from stem bark and leaves.

### 3.2. Studies on purified polysaccharide fractions

The crude extracts were further purified by ion exchange chromatography and the isolated sub-fractions with activity in the complement fixation assay, were subjected to gel filtration. The purified fractions thus obtained where the objects for further studies as given below.

#### 3.2.1. Yields

Two active purified polysaccharide fractions, TRBD-I-I and TRBD-I-II were obtained from the crude root bark extract, TRBD; one from stem bark crude extracts TSBD, called TSBD-II-I, but with higher yield. The leaf crude water extracts TLD gave two active fractions TLD-I-I and TLD-II-I. All of the yields given in Table 2 are based on the dried, powdered plant materials.

#### 3.2.2. Chemical composition

Chemical characterizations of the purified polysaccharide fractions are given in Table 2. They all contain the monosaccharides Ara, Rha, Gal, and GalA, being typical constituents in pectic polysaccharides. The monosaccharide compositions of the fraction TRBD-I-I were different from fraction TRBD-I-II, although they were isolated from the same native fraction TRB-I. TRB-I-I contains higher amount of Rha and GalA, while TRBD-I-II contains higher amount of Glc. The Glc present in TRBD-I-II most probably from starch, as positive reaction was observed in the iodine test. The monosaccharide compositions of TSBD-II-I were similar to that of TRBD-I-I, the main difference was found in GalA. The fractions TLD-I-I and TLD-II-I purified from TLD, contain quite different monosaccharide compositions, as no Xyl, Man and GlcA were detected in TLD-I-I. The fraction TLD-II-I was found to be rich in GalA (59.6 mol%), Ara (13.0 mol%) and Rha (10.6 mol%).

The positive Yariv test indicate the presence of AG-II in the root bark polysaccharide TRBD-I-I and in the purified active polysaccharide fractions from the stem bark and leaves.

The Bio-Rad protein assay showed negligible amounts ( $< 1\%$) of protein to be present in all fractions. A high amount of phenolic compounds was found in fraction TRBD-I-II (30.8%), determined by the Folin–Ciocalteu assay, while slight amounts were present in fractions TSBD-II-I, TLD-I-I and TLD-II-I.

#### 3.2.3. Molecular weight distribution

Size exclusion chromatography using dextran standards was employed to determine the average $M_W$ of the purified fractions. The highest molecular weight was found in the fraction TLD-I-I (115.9 kDa), followed by TRBD-I-I (71.5 kDa), while the lowest molecular weight was found in the fraction TRBD-I-II (5.5 kDa). Regarding the fractions from stem bark, fraction TSBD-II-I have a molecular weight being the same as that of the fraction TLD-II-I from leaves (23.3 kDa).

**Table 3**
The linkages (mol%) of the monosaccharides present in the purified fractions from root bark, stem bark and leaves of *Terminalia macroptera* determined by GC–MS after methylation.

| | | TRBD-I-I | TRBD-I-II | TSBD-II-I | TLD-I-I | TLD-II-I |
|---|---|---|---|---|---|---|
| Ara | T*f* | 6.3 | 7.9 | 6.4 | 18.8 | 5.7 |
| | 1,2*f* | 0.7 | 0.2 | 0.1 | 1.3 | 0.5 |
| | 1,3*f* | 1.2 | n.d. | 0.6 | 1.0 | 0.2 |
| | 1,5*f* | 4.7 | 3.1 | 2.3 | 9.8 | 4.0 |
| | 1,3,5*f* | 1.6 | 0.4 | 2.8 | 4.6 | 2.6 |
| Rha | T*p* | 2.5 | 5.8 | 3.1 | 0.8 | 1.5 |
| | 1,2*p* | 11.6 | 3.3 | 8.0 | 3.5 | 6.3 |
| | 1,2,4*p* | 3.0 | 1.1 | 2.0 | 1.0 | 2.2 |
| Xyl | 1,4*p* | 5.8 | 6.2 | 3.6 | 0.0 | 0.4 |
| Man | 1,3,6*p* | 2.7 | n.d. | 1.7 | n.d. | n.d. |
| Gal | T*p* | 3.4 | 4.5 | 4.5 | 5.3 | 1.8 |
| | 1,4*p* | n.d. | 0.8 | n.d. | n.d. | n.d. |
| | 1,3*p* | 3.4 | 1.0 | 1.6 | 5.3 | 1.9 |
| | 1,6*p* | 4.1 | 8.8 | 2.8 | 2.9 | n.d. |
| | 1,2,4*p* | 0.3 | 0.2 | 0.5 | n.d. | n.d. |
| | 1,3,6*p* | 7.8 | 1.8 | 3.2 | 15.5 | 4.1 |
| | 1,3,4,6*p* | 0.9 | 0.2 | 0.4 | 1.9 | 1.0 |
| Glc | T*p* | 0.8 | 9.2 | 0.4 | n.d. | n.d. |
| | 1,3*p* | 0.5 | 0.6 | 1.8 | 0.3 | n.d. |
| | 1,4*p* | n.d. | 8.5 | n.d. | 0.5 | 0.3 |
| | 1,6*p* | 0.4 | 8.2 | 0.8 | 0.1 | 0.8 |
| | 1,4,6*p* | n.d. | 0.6 | 1.0 | 0.2 | n.d. |
| GlcA | T*p* | 1.2 | 1.6 | 1.1 | n.d. | 0.9 |
| | 1,4*p* | 2.7 | 1.4 | 3.2 | n.d. | 4.5 |
| GalA | T*p* | 0.6 | n.d. | n.d. | n.d. | 1.9 |
| | 1,4*p* | 28.5 | 15.3 | 41.5 | 24.9 | 54.2 |
| | 1,3,4*p* | 3.3 | 5.4 | 4.7 | 1.4 | 3.6 |

### 3.2.4. Complement fixation activity

As can be seen from Fig. 3, the purified polysaccharide fractions showed potent human complement fixation activities *in vitro*. After purification, TRBD-I-I from the root bark showed higher activity compared to the positive control BPII. Fractions TRBD-I-II, TSBD-II-I, TLD-I-I and TLD-II-I showed similar activity, but lower than BPII.

### 3.2.5. Linkage analysis of the polysaccharide fractions

In order to determine the nature of the glycosidic linkages of the different monosaccharides in the purified fractions, permethylation of the reduced polymers was performed, partially O-methylated alditol acetates (PMAAs) were prepared and subjected to GC–MS. The results are given in Table 3.

The main structural feature of all fractions are similar, having 1, 4-linked galacturonan, with a few branch points in position 3 of GalA. The Rha units are basically 1, 2-linked, with a few branch points on position 4. The low ratio of Rha (including 1, 2-linked and 1, 2, 4-linked Rha) to GalA (including 1, 4-linked and 1, 3, 4-linked GalA) indicated that the backbone of the polysaccharide fractions consist of shorter RG-I structures, and longer homogalacturonan regions. The Gal and Ara present have the normal type of linkages that are found in the AG-II side chain (Paulsen et al., 2014). The Xyl is present as 1, 4-linked unit. These features have certain similarities with pectins that are composed of areas with hairy or ramified and smoother regions (Vincken et al., 2003).

TRBD-I-I contain longer RG-I region compared with the other purified fractions, since a higher ratio of 1, 2 Rha to 1, 4-linked GalA was found in TRBD-I-I. The presence of 1, 3-linked Gal and 1, 3, 6-linked Gal indicate the presence of AG-II structures in fractions TRBD-I-I, TSBD-II-I, TLD-I-I and TLD-II-I, also showed by positive reactions in the Yariv test. The occurrence of AG-II in the fraction TRBD-I-II could not be detected by the Yariv-test although 1, 3, 6 –linked Gal was present. This may be due to the fact that too short chains of 1, 3 linked Gal is present in this polymer (Paulsen, et al., 2014). 1, 4-linked Gal was only found in the fraction TRBD-I-II which may indicated the presence of the AG-I in this fraction only (Paulsen and Barsett, 2005).

GlcA appeared in the purified polysaccharide fractions (not detected in TLD-I-I) mainly as 1, 4-linked units, and with smaller amounts of terminally linked units. Terminal GlcA might be directly linked to position 3 of 1, 4-linked GalA in the RG-I backbone, or may also be a part of the AG-II side chains (Capek et al., 1987; Renard et al., 1999).

## 4. Discussion

In Mali, root bark, stem bark and leaves of *Terminalia macroptera* are used against a variety of ailments, such as sores and wounds, pain, cough, tuberculosis and hepatitis (Pham et al., 2011a). Medicinal plants used for wound healing often appear to be rich in polysaccharides, which may be responsible of their wound healing properties (Paulsen, 2001). The traditional healers in Mali prepare their herbal medicines by making water decoctions of the plant material. This is comparable to the BWE extraction procedure presented in this paper. Nosalova et al. (2013) found one pectic polysaccharide fraction from water extracts of *Terminalia chebula*, and showed potent antitussive activity. These results also support the biological activity of water extracts of medicinal plants. It was of interest to compare the structure and biological activity of polysaccharides from root bark, stem bark and leaves of *Terminalia macroptera* obtained by BWE.

The chemical and biological characteristics of crude extracts from different plant parts were similar, but had also some differences. The crude extract TRBD from root bark showed the highest activity, followed by TSBD and TLD. The root bark gave higher total complement fixation activities than stem bark and leaves in crude extracts obtained by BWE. The crude extracts TRBD, TSBD and TLD were fractionated by ion exchange chromatography and gel filtration as described, and led to the isolation of five active sub-fractions with different molecular weights. The chemical compositions of these sub-fractions were quite different (Table 2), but all contained monosaccharides typical for pectic type polysaccharides.

It has been reported that acidic polysaccharides with higher molecular weights appear to be more active in the complement assay than those with lower molecular weights (Grønhaug et al., 2010; Nergård et al., 2005; Togola et al., 2008). Among the five sub-fractions in our present study, the highest molecular weight fraction, TLD-I-I exhibited the lowest activity. The second higher molecular weight fraction, TRBD-I-I showed the highest activity.

In addition to molecular weight differences, the type of glycosidic linkages might be another reason for the influence on complement fixation activity. Pectins are generally known to be composed of linear homogalacturonan (HG) regions and branched rhamnogalacturonan (RG) I and II regions (Waldron and Faulds, 2007). The side chains of RG-I consist usually of arabinogalactan (AG) type I and/or II, as well as arabinan and galactan. Polysaccharides rich in AG-II have shown effects in a number of biological assays (Grønhaug et al., 2010; Paulsen and Barsett, 2005; Yamada and Kiyohara, 1999, 2007). Previously, RG-I containing polysaccharide with side chains of AG-I and/or AG-II have shown high complement fixation activities (Inngjerdingen et al., 2006). The results of the Yariv-test showed that all purified active fractions contain AG-II structures, except TRBD-I-II from the root bark. The fraction TLD-I-I is higher ramified compared to other fractions. As the RG-I backbone generally consists of alternating units of Rha and GalA, the low Rha to GalA ratio indicates that the backbone of the polysaccharide fractions consist of shorter RG-I structures, and longer homogalacturonan regions. As mentioned above, RG-I

regions were present in all fractions, but with different lengths. Sub-fraction TRBD-I-I contains longer RG-I region than other sub-fractions, as the linkages shown in Table 3. This may explain some of the higher complement fixation activity in TRBD-I-I than other sub-fractions. Sub-fraction TRBD-I-II may contain AG-I due to presence of 1, 4-linked Gal units, while absence of 1, 4-linked Gal indicated the absence of AG-I in other sub-fractions. TRBD-I-II contains also a high amount of Glc most probably from starch, as positive reactions were observed in the iodine test. This could be due to a RG-I backbone with a few Gal units attached in addition to starch, that bound physically tightly to the polymer and could not be removed by the method used.

Various immunomodulating polysaccharides isolated from plants (*Opilia celtidifolia*, *Vernonia kotschyana, Brassica oleracea*) also contain protein and phenolic compounds (Inngjerdingen et al., 2012; Samuelsen et al., 2007; Togola et al., 2008). The sub-fractions TRBD-I-II and TSBD-II-I contain higher amounts of protein and phenolic compounds than the other sub-fractions, which could explain some of the observed complement fixation activity in the lower molecular weight sub-fractions. Many phenolic compounds were shown to have potent complement activity, as reviewed by Pieters et al. (1999). The presence of phenolic substances in the purified fractions might be due to ferulic acid being linked as ester to Ara and Gal in pectins (Levigne et al., 2004).

The complement system is an important part of the innate immune system which also cooperates with the adaptive immune system in many ways (Dunkelberger and Song, 2010). The complement fixation assay has been used for a long time as a screening system for the interaction with the human immune system. It is a quick, highly reproducible assay performed in microtiter plates with many samples analyzed simultaneously. The indicator system in the assay is inhibition of hemolysis induced by human complement. Samples showing inhibition in the assay is thus having a direct effect on the human immune system (Michaelsen et al., 2000). Complement among other things play a direct part in the defense, such as primary defense against bacterial invasions and viral infections. Therefore, the traditional use of this tree to against wounds and various infection diseases may be connected to the complement system. The polysaccharides show potent complement fixation activities thus are, at least partly, response for the traditional use of this tree.

## 5. Conclusion

In summary, the crude extracts from root bark obtained by BWE showed higher total complement fixation activities than those from stem bark and leaves. Five purified polysaccharide fractions were isolated from TRBD, TSBD and TLD. These fractions had slightly different monosaccharide compositions, linkages and molecular sizes, but all contain monosaccharides typical for pectic polysaccharides. All purified fractions exhibited potent human complement fixation activities. The highest complement fixation activity was found in fraction TRBD-I-I, containing an RG-I backbone with side chains of AG-II. Quite high amount of protein and phenolic compounds was present in the fractions TRBD-I-II and TSBD-II-I and may explain some of detected activities. Both the root bark and stem bark are good sources for fractions containing bioactive polysaccharides. But due to sustainability, it is preferred to use leaves rather than the other two plant parts, but then the dosage by weight must be higher when using leaves.

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